

ABSTRACT

Disclosed is a method for determining the number of repeat units in a repeat region of a target nucleic acid. In a first aspect, the method of the invention includes the steps of annealing a primer to a target nucleic acid; performing a first primer extension reaction using a first primer extension reagent; separating the target-primer hybrid and unreacted first primer extension reagent; performing a second primer extension reaction using a second primer extension reagent, wherein at least one of the first or second primer extension reagents includes an extendible nucleotide having a label attached thereto; separating the target-primer hybrid from unreacted second primer extension reagent; measuring a signal produced by the label; treating the label so as to render the label undetectable; and repeating the above steps until the signal is substantially less than a signal detected in a previous cycle. In a second aspect, the method of the invention includes the steps of annealing a primer to a target nucleic acid; performing a first primer extension reaction using a first primer-extension reagent; separating the target-primer hybrid from unreacted first primer extension reagent; performing a second primer extension reaction using a second primer extension reagent and with a primer termination reagent, the primer termination reagent including a nucleotide terminator having a label attached thereto; separating the target-primer hybrid from unreacted second primer extension reagent and unreacted primer termination reagent; measuring a signal produced by the label; and repeating the above steps until a signal is detected indicating incorporation of the nucleotide terminator. The invention further includes kits useful for practicing the above methods.

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